REMARKS

Claims 7-32 are presently pending in this application and stand variously rejected under 35 U.S.C. §§ 112, 102 and 103. Applicants note with appreciation that the rejections not reiterated are withdrawn.

Claim 7 has been amended herein in a sincere effort to improve clarity. In particular, the terms "corresponding to" and "Ala-72" have been removed. In addition, claims 30, 31 and 32 have been rewritten in independent form. No new matter has been added as a result of these amendments and entry thereof is respectfully requested.

Written Description Rejections/New Matter Objections

Claims 7-32 stand rejected under 35 U.S.C. § 112, first paragraph as allegedly containing subject matter which was not adequately described in the specification. In particular, the Office assert that the specification does not describe SEQ ID NOs:1-4 or Figure 12 because the incorporation by reference of Domenighini was improper. (Final Office Action, pages 3-4). In addition, it is maintained that there is not an adequate written description in the specification of the term "fragments." (Final Office Action, page 6). For the Examiner's convenience, a copy of Domenighini is submitted herewith.

New Matter

The Examiner has objected to the claims, Figure 12 and the Abstract as allegedly containing new matter not described in the original specification. In support of these new matter rejections, the Office Action alleges, that neither the specification nor the original claims support for the recitation of SEQ ID NOs:1-4, the sequence shown in Figure 12 and the text of the Abstract. (Final Office Action, page 4 and 6). With regard to the reference to Domenighini and inclusion of sequences from this reference, it is further alleged that Applicants are not permitted to amend the specification because there is no specific statement regarding incorporation by reference of Domenighini in its entirety (see, Final Office Action, page 4, citing *In re de Seversky*, 177 USPQ 144 (CCPA 1973).

The Examiner has erred in determining that the claims, Sequence Listing, Figure 12 and the Abstract include new matter.

The proscription against the introduction of new matter in a patent application (35 U.S.C. 132 and 251) serves to prevent an applicant from adding information that goes beyond the

subject matter originally filed. See, *e.g., In re Rasmussen*, 650 F.2d 1212, 1214, 211 USPQ 323, 326 (CCPA 1981) and MPEP § 2163.06. Further, the claims as filed in the original specification are part of the disclosure. Therefore, if an application as originally filed contains a claim disclosing material not found in the remainder of the specification, the applicant may amend the specification to include the claimed subject matter. *See, e.g., In re Benno*, 768 F.2d 1340, 226 USPQ 683 (Fed. Cir. 1985).

In the pending case, the alleged "added" information does not in any way go beyond the subject matter originally filed. With regard to both Figure 12 and the sequences presented in the claims, Applicants note that the originally filed application specifically references Domenighini et al. (1995) for its disclosure of porcine and human sequences containing an Alanine at residue 72 (where the 72 refers to the position of the "correct" sequence presented in Figures 1 and 2 of Domenighini and added as Figure 12 herein). In addition, the originally filed claims also specifically referred to the Ala-72 residue of Domenighini as found in the amended claims and specification. Similarly, Domenighini also includes sequences presented in Figure 12 and as SEQ ID NOs:2-4 of the Sequence Listing. Finally, with regard to the Abstract, it was taken verbatim from PCT publication WO 98/18928, from which the pending application claims priority. Thus, there is nothing in the previous amendments that goes beyond the originally filed subject matter and, accordingly, new matter has not been added.

Applicants also disagree with the Examiner's contention that Domenighini was not properly incorporated by reference into the application. (See, pages 3-4 of the Final Office Action). The proper legal standard for determining if a reference is "properly" cited is not determining whether the words "incorporation by reference" appear next to the reference. Rather, the proper test involves examining the application for the context in which the reference is cited. *See, e.g.,* MPEP 608.01(p) and *In re Hawkins,* 179 USPQ 157 (CCPA 1973). Thus, mere reference, for example by listing of a number of references without pointing to specific teachings in these references or by simply using the words "continuation in part," is not a "proper" incorporation of these references. See, *In re de Seversky*, 177 USPQ 144 (CCPA 1973). In contrast, citing a reference for specific teachings in a specific context is not a "mere" reference. Indeed, the Federal Circuit has recently held that mere mention of the *title* of a journal article could be sufficient to describe claims in view of the understanding of the skilled artisan. *Atmel Corp. v. Information Storage Devices Inc.,* 53 USPQ2d 1225, 1231 (Fed. Cir. 1999).

Here, Applicants properly referenced Domenighini et al. in the specification on page 5. Moreover, this identification of Domenighini is entirely for specific teachings regarding the sequence of various LT-A proteins and, additionally, for alignment of these various sequences showing the position of amino acids relative to the wild-type Ala-72 in the porcine LT-A protein. Thus, Applicants have properly amended the specification to include sequences from these references and from sequences publically available at the time of filing.

Even assuming, for the sake of argument only, that Domenighini was not properly referenced, it is also well-settled that applicants must be afforded an opportunity to correct incorporate material deemed essential into their specification. *See, e.g.,* M.P.E.P. 608.01(p)(I)(A)(2). Indeed, in *Hawkins,* the CCPA made clear that subsequent amendment of the specification to recite the teachings of the reference will properly cure any defect in disclosure alleged by the Office and that editing the application by inserting that which was previously properly referenced does not raise new matter issues. *In re Hawkins,* 179 USPQ at 161. Here, the Examiner maintains that the teachings in Domenighini are essential and that they were not properly incorporated by reference. By inserting the sequences of Domenighini into the specification, Applicants have entirely cured the alleged defect. In this regard, Applicants also note that the sequences of Domenighini and those added to the specification were publically available at the time of filing. (See, *e.g.,* attached GenBank entry).

Finally, Applicants also traverse the Examiner's assertion that the sequence presented as SEQ ID NO:1 differs from that presented in Domenighini or from the sequences that were publically available at the time of filing. Applicants can find no difference as between SEQ ID NO:1 (as claimed) and the "correct" sequences presented in Figures 1 and 2 of Domenighini. Further, even if certain amino acids were different as between Domenighini and Figure 12, it is well known that sequencing errors are a common problem in molecular biology. See, e.g., Peter Richterich, Estimation of Errors in 'Raw' DNA Sequences: A Validation Study, 8 Genome Research 251-59 (1998). Any discrepancies in sequence between Domenighini and Figure 12 fall under the category of "minor errors" that can readily be corrected. Indeed, prior to the filing of the present application, the sequence of LT-A was updated in January, 1996. (See, attached GenBank entry). Further, the GenBank entry also indicates that this is not an instance in which a deposit was made after the filing date of the application. Rather, the GenBank entry indicates that the starting wild-type LT-A sequences was available at the time of filing.

Thus, Applicants submit that the amendments to the claims and to the specification do not constitute the addition of new matter. Nonetheless, if the Examiner prefers, Applicants would be willing to incorporate the sequence directly into the specification following the reference to Domenighini.

Written Description

Applicants also traverse the rejection of claims 7-29 on the grounds that the specification allegedly does not describe "fragments in such detail that will sufficiently identify the epitope sequence." (Final Office Action, page 6). In further support of this rejection it is stated:

> [T]he cited description merely states that a fragment should contain Ala residue (which is further replaced). Location of this residue is irrelevant because only a single residue is identified and thus any Ala residue will satisfy this structural requirement as no other structural characteristics (e.g., the nature of other residues present in the fragment and their relation to the sequence of LT-A) are identified. There is no guidance on core structure of said fragment which would render a "immunological fragment" as claimed. The mere presence of arginine (i.e., the residue substituting Ala) is not sufficient for identification of a core structure. As there are no sufficient structural characteristics for DNA encoding thereof, and correspondent vectors, host cells and uses thereof. (Final Office Action, page 6).

The fundamental factual inquiry in written description is whether the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, applicant was in possession of the invention as now claimed. *See, e.g.*, Vas-Cath, Inc., 935 F.2d at 1563-64, 19 USPQ2d at 1117. The burden is on the Examiner to provide evidence as to why a skilled artisan would not have recognized that the applicant was in possession of claimed invention at the time of filing. *See, e.g., In re Edwards*, 196 USPQ 465, 469 (CCPA 1978). No such evidence has been provided. Accordingly, Applicants traverse the rejection and supporting remarks.

Applicants reiterate that the claims require more structural limitations than the "mere presence" of arginine. Rather, claims 7-29 each require that the polypeptide encoded by the polynucleotide is an immunologically effective detoxified fragment of at least 8 amino acids in length and that this fragment contain a mutation in residue 72, as numbered relative to SEQ ID NO:1. As previously noted the specification further describes how these fragments of LT-A must include an arginine at residue 72, numbered relative to SEQ ID NO:1 (Domenighini). (See,

e.g., page 5, lines 14-15). It is well within the purview of the skilled artisan, in view of the teachings of the specification, to construct fragments of LT-A containing residue 72 (numbered relative to SEQ ID NO:1) and to replace the wild-type residue with an arginine. (see, *e.g.,* page 5, lines 14-15; page 17, lines 10-19 and Examples of the specification). These fragments could readily be tested for toxicity (*e.g.,* in the well known Y1 cell assay). (see, *e.g.,* page 43. line 35 to page 44 line 21 for example of Y1 assay). Thus, the specification clearly conveys to a skilled artisan that applicants were in possession of the precisely claimed molecules at the time the application was filed.

With respect to the description of nucleotide sequences encoding these proteins, Applicants direct the Examiner's attention to page 41, lines 35-38 of the specification, describing sources of wild-type LT-A-encoding sequences (*e.g.,* citing Pronk et al and Spicer et al.). Further, it is well within the purview of a skilled artisan to align sequences with SEQ ID NO:1; to determine which residue corresponds to Ala-72; and how to substitute arginine for this residue. (See, *e.g.,* page 42 of the specification). Methods of including these polynucleotides in vectors, host cells and the like are similarly described in the specification and within the purview of the skilled artisan. (See, *e.g.,* pages 6-8 and 19 to 43 of the specification). Accordingly, in light of Applicants' disclosure and state of the art at the time of filing, designing and using nucleic acid molecules encoding the claimed LT-A mutants is well within the purview of a skilled artisan.

Applicants further submit that the Rule 132 Declaration of Dr. del Giudice submitted on in the parent case has not been adequately considered or in any way rebutted. It is well established that declaratory evidence must be considered. *In re Alton,* 37 USPQ2d 1578 (Fed. Cir. 1996). In *In re Alton,* The Court of Appeals for the Federal Circuit held that it was error for the Examiner to dismiss, with conclusory statements, not only factual statements but also statements of opinion presented in Declarations made by qualified persons of ordinary skill in the art. The Federal Circuit also commented that they were "aware of no reason why opinion evidence relating to a fact issue should not be considered by an Examiner." 37 USPQ2d 1578 at 1583 n10.

Applicants have provided ample factual evidence which demonstrates that the specification as filed describes the subject matter of the pending claims. The Office in the pending case has failed to adequately consider and rebut the facts and reasoned conclusions

presented in the del Giudice Declaration. When properly considered, the Declaration of record in the parent application clearly establish that fragments are described in the specification. In fact, Dr. del Giudice completely rebuts the Examiner's conclusions. The Declaration sets forth certain factual evidence upon which Dr. del Giudice bases a reasoned opinion. In particular, Dr. del Giudice states the following facts.

First, at the time the specification was filed, the sequences of various wild-type LT-A polypeptides were known and publically available. (See, del Giudice Declaration, paragraph 5).

Second, the specification teaches how to obtain detoxified mutants of wild-type LT-As in which a particular residue of the wild-type polypeptide is substituted with an arginine residue. (See, del Giudice Declaration, paragraph 5).

Third, the specification teaches how to obtain fragments of these detoxified mutants, where the fragments that contain an arginine at residue 72, numbered relative to the correct sequences presented in Domenighini. (See, del Giudice Declaration, paragraph 6).

Fourth, the specification describes how to test these mutants for immunogenicity. (See, del Giudice Declaration, paragraph 6).

Dr. del Giudice uses these facts and other facts to come to conclude:

> 6. It is also my opinion that one working in this field would have expected functional fragments of the full-length LT-R72 to be immunogenic. The application indicates that such fragments must contain the Ala-72 residue and that these functional fragments are preferably at least 8 amino acids in length. (page 5, lines 14-15 and page 17, lines 10-19 of the application). Thus, is my further opinion that a scientist working in the field could have constructed, using conventional methods known in the art in combination with the teachings of the specification, immunogenic polypeptides which (1) were fragments of full-length LT-A; (2) contained sequence corresponding to the Ala-72 residue; and (3) were at least 8 amino acids in length. Furthermore, a scientist would have been readily able to test such constructs for immunogenicity; toxicity (*e.g.*, in the well known Y1 cell assay); and for their effectiveness as mucosal adjuvants (*e.g.*, following the guidelines of the application). Thus, I believe that the application as filed clearly conveys to a skilled artisan that the inventors were in possession of the claimed LT-A fragments at the time the application was filed. (See, Declaration and attached Exhibits submitted in parent case).

Thus, using specific facts, Dr. del Giudice arrives at the conclusion that the application as filed clearly conveys to the skilled artisan that Applicants were in possession of the claimed

invention at the time of filing. This convincing, factual evidence has been improperly ignored by the Office (*see, e.g., In re Alton,* 37 USPQ2d 1578, 1584 (Fed. Cir. 1996)).

Indeed, by summarily dismissing the Declaratory evidence provided by Applicants and maintaining the written description rejection without modification, the Examiner is substituting personal knowledge for that of Dr. del Giudice. When a rejection is based on facts within the personal knowledge of the Examiner, the data relied upon should be stated as specifically as possible, and the reference must be supported, when called for by the applicant, by an affidavit from the Examiner. 37 C.F.R. 1.104(d)(2); MPEP 2144.03. Applicants again request such an affidavit.

In view of the foregoing amendments and remarks, Applicants submit that the pending claims are fully described by the specification as filed and respectfully request that the rejection be withdrawn.

Rejections Under 35 U.S.C. § 112, First Paragraph, Enablement

Claims 7-29 stand rejected as allegedly not enabled by the specification as filed. In support of this rejection it is maintained:

> As there is a plurality of LT-A proteins and only proteins having a replaced Ala residue in position 72 are subject of invention. However, no particular LT-A having sequence with Ala or Arg in the indicated position 72 is included in the claims or enabled by the disclosure. Multiple descriptions of the prior art cited in the specification, p. 5, line 25 +, describes LT-A proteins having not Ala72, but a residue "which corresponds to Ala72". However, no particular LT-A having sequence with Ala in the indicated position is included in the claims or is present in the specification. Accordingly, the claims and the specification lack the essential subject matter. See *In re Mayhew,* 188 USPQ 356 (CCPA 1976).

The test of enablement is whether one of skill in the art could make and use the invention based on the specification as a whole. A specification must be taken as enabling in the absence of evidence to the contrary. The courts have consistently held that not every last detail of any invention need be described, "else patent specifications would turn into production specifications, which they were never intended to be." See, e.g., *In re Gay*, 309 F.2d 769, 774 135 USPQ 311, 316 (CCPA 1962) and *Staehelin v. Secher* 24 USPQ2d 1513, 1516 (BPAI 1992).

Moreover, the Office must consider evidence provided by the applicant that one skilled in the art would be able to make and use the claimed invention using the application as a guide.

See, e.g., PTO Training Manuals on Enablement, page 42; MPEP 716.09; *In re Brandstadter,* 179 USPQ 286 (CCPA 1973); *In re Ambruster,* 185 USPQ 152 (CCPA 1975); and *In re Alton,* 37 USPQ2d 1578, 1584 (Fed. Cir. 1996). The evidence provided by the applicant need not be conclusive but merely convincing to one skilled in the art. PTO Training Manual on Enablement, page 42. Thus, Applicants are under no legal obligation to exemplify each and every member of a claimed genus. Rather, for a claimed genus, representative examples together with a statement applicable to the genus as whole is sufficient to establish enablement if the skilled artisan would expect the claimed genus could be used in the manner set forth. *See, e.g.,* U.S. Patent and Trademark Office's Training Materials on Enablement, p. 29. Thus, the proper legal standard for determining enablement is whether the specification provides enough guidance as to the existence of methods and materials that allow one of skill in the art to practice the claimed invention without undue experimentation. (see, *e.g., In re Wands,* 8 USPQ2d at 1404, citing *In re Angstadt,* 190 USPQ 214 (CCPA 1976)).

The present record plainly establishes one of skill in the art could make and use the claimed molecules without undue experimentation following the guidance set forth in the specification as filed. As previously pointed out, the specification is replete with representative examples and statements applicable to designing, making and testing nucleotides encoding detoxified LT-A polypeptides as claimed. The specification is also replete with descriptions of how to align any given LT-A polypeptide with SEQ ID NO:1 to determine which amino acid residue corresponds to Ala-72 (and, indeed, Domenighini and Figure 12 show such alignments). The specification also described, in detail, how to replace this residue with an arginine and how to test for detoxification and for immunogenicity. (See, Examples). In addition, the Examples also detail how to use the mutants, for instance as adjuvants. Further evidence establishing enablement is also of record in the form of a Rule 132 Declaration submitted in the parent application. Therefore, the evidence demonstrates that the specification fully enables the pending claims.

The Office's reliance on *In re Mayhew,* 188 USPQ 356 (CCPA 1976) is entirely misplaced. *In re Mayhew* was directed to the issue of whether the claims failed to recite a necessary limitation. This is not the issue in the case at hand where the pending claims clearly recite that the claimed molecule encodes a detoxified LT-A fragment of at least 8 amino acids in length, that the polypeptide includes residue 72 (numbered relative to SEQ ID NO:1) and that

this residue is an arginine. Nor does *Mayhew,* after 26 years, outweigh the many Federal Circuit and CCPA decisions holding that the specification is considered presumptively enabling of the claims. In the pending case, the Office has not shown, with evidence, reasons as to why the specification is not enabling. Accordingly, a *prima facie* case of non-enablement has not been established and withdrawal of this rejection is respectfully requested.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 7–32 stand rejected as allegedly indefinite for the reasons set forth below. Applicants address each rejection in turn.

Claim 7 stands rejected as allegedly ambiguous "in relation to the structure of the product claimed." (Final Office Action, page 7). In particular, it is alleged that there are conflicting recitations as to whether Ala-72 is included or excluded. (Final Office Action, page 7). Although Applicants submit that the previous claim language was both clear and definite, claim 7 has been amended herein to remove the terms "corresponding to" and "Ala-72." The claim has also been reformatted for clarity. In view of the foregoing amendments, Applicants respectfully request that this rejection be withdrawn.

Claims 30-32 stand rejected as allegedly confusing in that they encompass human and/or porcine sequences. Claims 30-32 have been rewritten herein in independent format. Further, as detailed above, Figure 12 and the accompanying Sequence Listing are properly included in the application based on the specific reference to Domenighini for its disclosure and alignment of porcine and human LT-A sequences.

Rejections Under 35 U.S.C. § 103(a)

Claims 7-29 are rejected under 35 U.S.C. § 103(a) as allegedly obvious over EP145486. It is maintained that this reference teaches the compositions and vaccines comprising modified LT-A that includes an 8 amino acid fragment including the Ala-72 substituted residue. (Final Office Action, page 8, underlining residues 67-75 of the sequence shown).

Applicants traverse the rejection and supporting remarks.

The pending claims are directed to polynucleotides encoding detoxified fragments of LT-A mutant polypeptides in which the mutation is in the amino acid residue numbered 72 in SEQ ID NO:1 (also the uppermost line of Figure 12). This mutated residue must be included in the claimed polypeptide. The reference cited by the Office does not in any way teach or suggest

such molecules. The fact that EP145486 contains an arginine at its position 72 is irrelevant because this residue is not position 72 as numbered relative to SEQ ID NO:1. In fact, the cited sequence does **not** include residue 72, numbered relative to SEQ ID NO:1, as required by the pending claims. To further illustrate this point, SEQ ID NO:1 is reproduced below. The sequence from the reference cited by the Office is underlined while the residue that must be included (and must be an arginine) in the claimed molecules is shown in bold:

SEQ ID NO:1 (FIG. 12):

NGDRLYRADSRPPDEIKRSGGLMPRGHNEYFDRGTQMNINLYDHARGTQTGFVRYDDGYVSTSLS
LRSAHLAGQSILSGYSTYYIYVIATAPNMFNVNDVLGVYSPHPYEQEVSALGGIP
YSQIYGWYRVNFGVIDERLHRNREYRDRYYRNLNIAPAEDGYRLAGFPPDHQAWREEPWIHHAPQ
GCGNSSRTITGDTCNEETQNLSTIYLREYQSKVKRQIFSDYQSEVDIYNRIRDEL

Simply put, the fragment disclosed in EP145486 does not include residue 72, numbered relative to SEQ ID NO:1 and, accordingly, the fragment does not fall within the scope of the pending claims. Nor does EP 145486 describe, demonstrate or suggest mutated LT sequences in which the wild-type alanine residue at position 72 of SEQ ID NO:1 is substituted with arginine.

Therefore, the cited reference fails to teach or suggest the precisely claimed invention and, accordingly, withdrawal of the rejections based on this reference is respectfully requested.

Rejections Under 35 U.S.C. § 102(e)

Claims 7-29 are rejected under 35 U.S.C. § 102(e) as allegedly anticipated by U.S. Patent No. 5,770,203 (hereinafter "Burnette"). (Final Office Action, page 10). In particular, Burnette is alleged to disclose fragment 29-54 of SEQ ID NO:1 which includes various Arginine residues. (Final Office Action, page 10).

Because Burnette does not describe or disclose the claimed molecules, Applicants traverse.

In order to be an anticipatory reference, the single reference cited by the Office must disclose each and every element of the claims. *Hybritech v. Monoclonal Antibodies,* 231 USPQ 81 (Fed. Cir. 1986). In the pending case, Burnette fails to disclose a detoxified LT-A polypeptide and a fragment of a detoxified LT-A polypeptide of at least 8 amino acids in length, where the fragment includes residue 72, numbered relative to SEQ ID NO:1. Burnette also fails to disclose such a fragment in which residue 72, numbered relative to SEQ ID NO:1, is an

arginine (rather than the wild-type residue). This is further illustrated below, where Burnette's sequence is underlined and does not include the bolded residue required by the claims:

SEQ ID NO:1:

NGDRLYRADSRPPDEIKRSGGLMPRGHNEYFDRGTQMNINLYDHARGTQTGFVRYDDGYVSTSLS
LRSAHLAGQSILSGYSTYYIYVIATAPNMFNVNDVLGVYSPHPYEQEVSALGGIP
YSQIYGWYRVNFGVIDERLHRNREYRDRYYRNLNIAPAEDGYRLAGFPPDHQAWREEPWIHHAPQ
GCGNSSRTITGDTCNEETQNLSTIYLREYQSKVKRQIFSDYQSEVDIYNRIRDEL

Thus, the cited reference does not describe, demonstrate or suggest the claimed molecules – all of which include a polynucleotide encoding a detoxified LT-A polypeptide that includes an arginine at amino acid residue 72, numbered relative to SEQ ID NO:1. Withdrawal of this rejection is respectfully requested.

CONCLUSION

In view of the foregoing, Applicant submits that the claims are now in condition for allowance and requests early notification to that effect.

Please direct all further communications regarding this application to:

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

7. (Twice Amended) A polynucleotide encoding an immunologically effective detoxified fragment of an *E. coli* heat labile toxin (LT-A) polypeptide, wherein

(i) said fragment is at least 8 amino acids in length;

(ii) said fragment includes an amino acid residue at position 72, numbered relative to SEQ ID NO:1; and

(iii) said amino acid residue at position 72, numbered relative to SEQ ID NO:1, is an arginine residue [and further wherein the said fragment comprises the amino acid residue corresponding to Ala-72 of SEQ ID NO:1 and wherein said residue is substituted with an arginine residue].

30. (Amended) [The polynucleotide of claim 7, wherein the fragment comprises amino acid residues of SEQ ID NO:2.] A polynucleotide encoding an immunologically effective detoxified fragment of an *E. coli* heat labile toxin (LT-A) polypeptide, wherein

(i) said fragment is at least 8 amino acids in length;

(ii) said fragment includes the amino acid residue at position 72, numbered relative to SEQ ID NO:2; and

(iii) said amino acid residue at position 72, numbered relative to SEQ ID NO:1, is an arginine residue.

31. (Amended) [The polynucleotide of claim 7, wherein the fragment comprises amino acid residues of SEQ ID NO:3.] A polynucleotide encoding an immunologically effective detoxified fragment of an *E. coli* heat labile toxin (LT-A) polypeptide, wherein

(i) said fragment is at least 8 amino acids in length;

(ii) said fragment includes the amino acid residue at position 72, numbered relative to SEQ ID NO:3; and

(iii) said amino acid residue at position 72, numbered relative to SEQ ID NO:1, is an arginine residue.

32. (Amended) [The polynucleotide of claim 7, wherein the fragment comprises amino acid residues of SEQ ID NO:4.] A polynucleotide encoding an immunologically effective detoxified fragment of an *E. coli* heat labile toxin (LT-A) polypeptide, wherein

(i) said fragment is at least 8 amino acids in length;

(ii) said fragment includes the amino acid residue at position 72, numbered relative to SEQ ID NO:4; and

(iii) said amino acid residue at position 72, numbered relative to SEQ ID NO:1, is an arginine residue.

PENDING CLAIMS

7. (Twice Amended) A polynucleotide encoding an immunologically effective detoxified fragment of an *E. coli* heat labile toxin (LT-A) polypeptide, wherein

(i) said fragment is at least 8 amino acids in length;

(ii) said fragment includes an amino acid residue at position 72, numbered relative to SEQ ID NO:1; and

(iii) said amino acid residue at position 72, numbered relative to SEQ ID NO:1, is an arginine residue.

8. The polynucleotide of claim 7 further comprising a sequence encoding a second immunogenic antigen.

9. The polynucleotide of claim 8 wherein the second immunogenic antigen comprises a subunit B of an *E. coli* heat labile toxin (LT-B).

10. The polynucleotide of claim 9, wherein the LT-A and LT-B are encoded in a polycistronic unit.

11. An expression vector comprising the polynucleotide of claim 7.

12. An expression vector comprising the polynucleotide of claim 8.

13. An expression vector comprising the polynucleotide of claim 9.

14. An expression vector comprising the polynucleotide of claim 10.

15. A host cell comprising the expression vector of claim 11.

16. A host cell comprising the expression vector of claim 12.

17. A host cell comprising the expression vector of claim 13.

18. A host cell comprising the expression vector of claim 14.

19. The host cell of claim 15, wherein the host cell is selected from the group consisting of a bacterium, a mammalian cell, a baculovirus, an insect cell and a yeast cell.

20. The host cell of claim 19, wherein the host cell is *E. coli.*

21. The host cell of claim 19, wherein the host cell is a mammalian cell.

22. The host cell of claim 19, wherein the host cell is an insect cell.

23. The host cell of claim 19, wherein the host cell is a yeast cell.

24. The host cell of claim 19, wherein the host cell produces the amino acid sequence intracellularly.

25. The host cell of claim 19, wherein the host cell secretes the amino acid sequence.

26. The *E. coli* host cell of claim 19, wherein the host cell is mutated to produce a phenotype lacking wild type LT-A.

27. A method of producing a recombinant protein comprising:

(a) providing a population of host cells according to claim 15; and

(b) culturing said population of cells under conditions whereby the LT-A or fragment thereof encoded by the polynucleotide in said expression vector is expressed.

28. A method of producing a recombinant protein comprising:

(a) providing a population of host cells according to claim 17; and

(b) culturing said population of cells under conditions whereby the LT-A or fragment thereof and the LT-B encoded by the polynucleotide in said expression vector is expressed.

29. A method of producing a recombinant protein comprising:

(a) providing a population of host cells according to claim 26; and

(b) culturing said population of cells under conditions whereby the LT-A or fragment thereof encoded by the polynucleotide in said expression vector is expressed.

30. (Amended) A polynucleotide encoding an immunologically effective detoxified fragment of an *E. coli* heat labile toxin (LT-A) polypeptide, wherein

(i) said fragment is at least 8 amino acids in length;

(ii) said fragment includes the amino acid residue at position 72, numbered relative to SEQ ID NO:2; and

(iii) said amino acid residue at position 72, numbered relative to SEQ ID NO:1, is an arginine residue.

31. (Amended) A polynucleotide encoding an immunologically effective detoxified fragment of an *E. coli* heat labile toxin (LT-A) polypeptide, wherein

(i) said fragment is at least 8 amino acids in length;

(ii) said fragment includes the amino acid residue at position 72, numbered relative to SEQ ID NO:3; and

(iii) said amino acid residue at position 72, numbered relative to SEQ ID NO:1, is an arginine residue.

32. (Amended) A polynucleotide encoding an immunologically effective detoxified fragment of an *E. coli* heat labile toxin (LT-A) polypeptide, wherein

(i) said fragment is at least 8 amino acids in length;

(ii) said fragment includes the amino acid residue at position 72, numbered relative to SEQ ID NO:4; and

(iii) said amino acid residue at position 72, numbered relative to SEQ ID NO:1, is an arginine residue.